DERWENT-ACC-NO: 1995-253643

DERWENT-WEEK: 199533

TITLE: Polysaccharide antigen and polyethyleneimine conjugate - useful as immuno-sorbent for detecting streptococcal and pneumococcal infections

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PATENT-ASSIGNEE: A MED SIBE CLINICAL EXPER MEDICINE[AMSIR] , VACCINE SERUM RES INST[VACCR]

PRIORITY-DATA: 1990SU-4859936 (August 22, 1990)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
RU 2027190 C1	January 20, 1995	N/A	005	G01N 033/531

APPLICATION-DATA:

PUB-NO	APPL-DESCRIPTOR	APPL-NO	APPL-DATE
RU 2027190C1	N/A	1990SU-4859936	August 22, 1990

INT-CL (IPC): G01N033/531

ABSTRACTED-PUB-NO: RU 2027190C

BASIC-ABSTRACT:

A conjugate of polysaccharide antigen and polyethylene imine as an immunosorbent for detecting streptococcal and pneumococcal infections of general formula (I) is new.

n = 103-104.

ADVANTAGE - This conjugate, in which polyethyleneimine is used as the bonding
linkage, avoids some disadvantages of previous conjugates, e.g. the use of bonding agents which are suitable only for protein antigens, which are insufficiently specific, and the use of an antigen-polysaccharide-protein conjugate, which has low adhesional properties, and can be used only with solid
activated carriers, e.g. polystyrene, PVC, polysaccharide.

CHOSEN-DRAWING: Dwg.0/0

TITLE-TERMS: POLYSACCHARIDE ANTIGEN POLYETHYLENEIMINE CONJUGATE USEFUL IMMUNO
SORPTION DETECT STREPTOCOCCUS PNEUMOCOCCUS INFECT

DERWENT-CLASS: A96 B04 S03

CPI-CODES: A03-A00A; A05-J07; A09-A08; A12-V03C2; B04-C03D; B04-F10B;
B12-K04A4;

EPI-CODES: S03-E14H4;

CHEMICAL-CODES:
Chemical Indexing M1 *01*
Fragmentation Code
F012 F013 F014 F015 F016 F123 H1 H100 H101 H181

H4 H401 H421 H5 H522 H8 J0 J011 J3 J321
K0 L1 L8 L818 L824 L835 M280 M322 M332 M342
M373 M383 M392 M423 M424 M510 M521 M530 M540 M710
M740 M903 M904 P831 Q120 Q132 V735 V743
Markush Compounds
199533-30501-D 199533-30501-N

Chemical Indexing M1 *02*
Fragmentation Code
M423 M750 M903 N102 Q120 Q132 V500 V540

Chemical Indexing M6 *03*
Fragmentation Code
M903 P831 Q120 Q132 R515 R521 R627 R635

ENHANCED-POLYMER-INDEXING:
Polymer Index [1.1]
017 ; G3703 G3623 P0599 D01 ; H0226 ; L9999 L2391 ; M9999 M2835 ; L9999 L2835
Polymer Index [1.2]
017 ; ND01 ; ND06 ; Q9999 Q7998 Q7987 ; B9999 B5094 B4977 B4740
Polymer Index [1.3]
017 ; D00 Na 1A O* 6A I* 7A ; H0226
Polymer Index [2.1]
017 ; R01176 G1650 G1649 D01 D23 D22 D31 D41 D50 D82 F08 F07 ; H0000
; H0226 ; P0055 ; P1116 P1105 D01 D10 F07 ; M9999 M2835 ; L9999 L2391 ; L9999 L2835
Polymer Index [2.2]
017 ; ND01 ; ND06 ; Q9999 Q7998 Q7987 ; B9999 B5094 B4977 B4740
Polymer Index [2.3]
017 ; H0226

SECONDARY-ACC-NO:
CPI Secondary Accession Numbers: C1995-116053
Non-CPI Secondary Accession Numbers: N1995-195739

PTO 2004-2466

Russian Patent

Document No. 2,027,190 C1

POLYSACCHARIDE ANTIGEN - POLYETHYLENEIMINE (PEI) CONJUGATE AS AN IMMUNOSORBENT FOR DETECTION OF STREPTOCOCCUS AND PNEUMOCOCCUS INFECTIONS

[KONYUGAT POLISAKHARIDNOGO ANTIGENA S POLIETILENIMINOM V KACHESTVE IMMUNOSORBENTA DLYA VYIAVLENIYA STREPTOKOKKOVYKH I PREVMOKOKKOVYKH INFEKTSIY]

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UNITED STATES PATENT AND TRADEMARK OFFICE

Washington, D.C. MAY 2004

Translated by: Schreiber Translations, Inc.

Country	:	Russia
Document No.	:	1,027,190 C1
Document Type	:	Patent description
Language	:	Russian
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IPC	:	G 01 N 33/531
Application Date	:	August 22, 1990
Publication Date	:	January 20, 1995
Foreign Language Title	:	Konyugat polisakharidnogo antigena s polietileniminom v kachestve immunosorbenta dlya vyiavleniya streptokokkovykh I pnevmokokkovykh infektsiy
English Title	:	POLYSACCHARIDE ANTIGEN - POLYETHYLENEIMINE (PEI) CONJUGATE AS AN IMMUNOSORBENT FOR DETECTION OF STREPTOCOCCUS AND PNEUMOCOCCUS INFECTIONS /1[1]

[1] Numbers in the margin indicate pagination in the foreign text.

ABSTRACT

Field of application of the invention: immunology; biotechnology. The object of invention: With our invented synthetic immunosorbent produced by treatment of polysaccharide antigen with sodium periodate and polyethyleneimine it becomes possible to detect streptococcus and pneumococcus infections. Our immunosorbent is approximately 300 million Daltons in molecular mass, and it is suitable for application in the agglutination tests for detection of pneumococcus and streptococcus infections. 2 tables.

References/Prototypes:

1. Patent USA No. 4,356,170; APC A 61K 39/385, 1982/2

POLYSACCHARIDE ANTIGEN - POLYETHYLENEIMINE (PEI) CONJUGATE/3

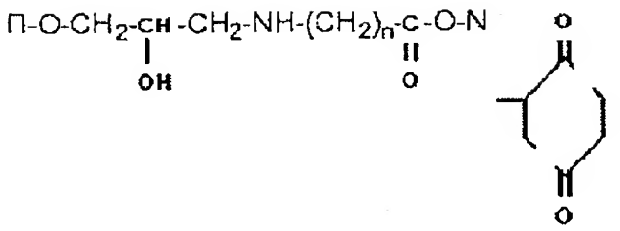
AS AN
IMMUNOSORBENT FOR DETECTION OF STREPTOCOCCUS AND PNEUMOCOCCUS

INFECTIONS

The invention pertains to immunobiotechnology, specifically to conjugates and immunosorbents.

Our invented immunosorbent may be applied for diagnostics of infectious inflammatory diseases.

There is a known activated matrix of general chemical formula



where П = residue of sucrose, Sefadex, or cellulose (n = 2 or 3 for immobilization of proteins) (A. A. Zenyuk et al. USSR Patent No. 1,280,834; IPC G 01 N 33/50).

The above inventors made use of polysaccharide matrices produced with a three-stage process as follows:

1. Activation of a polysaccharide carrier by epichlorohydrin.

2. Scission of the epoxy ring by an amino acid.

3. Etherification of the carboxyl by N-oxysuccinimide.

The proteins, which contain a primary amino group, e. g. Transcortin, a secosteroid that binds globulin and immunoglobulins, are immobilized on a matrix.

Such matrix, however, is unsuitable for immobilization of polysaccharide antigens: they are failing to bind the binding element applied.

Further, there is a known conjugate in which the protein antigen's is bonded either with a sulfonation or alkylating agent or with acylation agents (An Agglutination Reagent and Method of Its Production - Patent Application EPO (European Patent Office) No. 280,561; IPC G 01 N 33/546).

The above binding agents, however, are suitable only for protein antigens; not infrequently they are not adequately specific to detect illnesses thus reducing the accuracy of diagnostics.

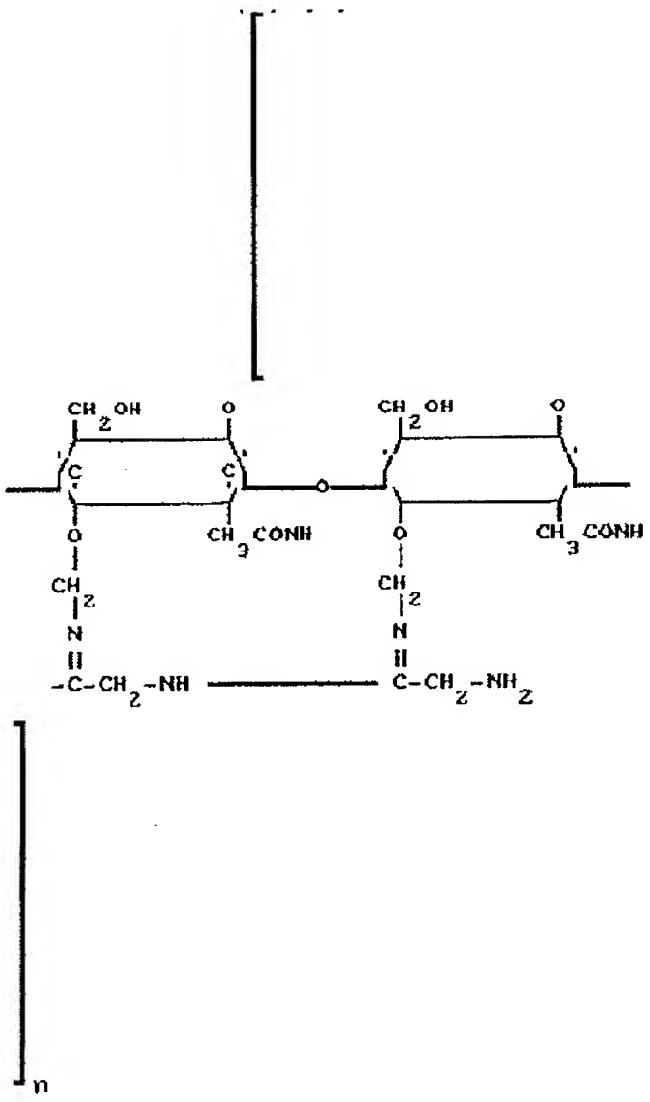
Moreover, there is a known immunosorbent [1] which we have selected as the prototype. This is an antigen-polysaccharide-protein conjugate; polysaccharide and protein here are bonded through a $-CH_2-H-$ protein. It is not possible to use this
N

immunosorbent in solutions due to its poor agglutination properties. Further, because of low adhesive capacity it may be applied exclusively with activated solid-phase carriers. In particular, the conjugate cannot be applied with any inactivated solid-phase carriers, e.g. those made of polystyrene, polyvinyl chloride or polysaccharide.

So, our challenge was to create a conjugate with good agglutination properties (it should be possible to use it in solutions) and also with adequate adhesive capacity (enough for its application with inactivate solid phases). To solve this task is to extend the range of conjugate's applications considerably.

Our engineering/technology finding is that we bind polyethyleneimine (PEI) to polysaccharide's aldehyde grouping.

The substance of our invention is that the conjugate has a general formula:



Where $n = 10^3 - 10^6$.

It is known from publications (See above) that polysaccharide is being applied as an antigen and as an activated carrier. However, there are no reported data on any application of polyethyleneimine as a binding unit. We only know about its applications as a polycation for binding of sulfo

groups, carboxyls, and also some other acidic groups. (See Haars A., Zonnez S., Hutterma A. Quantitative determination of lignosulfamates from sulfite spent liquors using precipitation with polyethyleneimine. Holzforsch 35 (2); 59 - 65. 81).

Thus it becomes obvious that we are the pioneers, and our findings are just an invention.

We have obtained our conjugate based on the following procedure:

A polysaccharide antigen, e.g. C-polysaccharide from pneumococcus or A-polysaccharide from Group A streptococcus was treated by sodium periodate; then polyethyleneimine solution was added. The entire schematic representation of the reaction is as follows

1. $(C_6H_{10}O_5)_n + 2$

$NaIO_4 \rightarrow (C_6H_6O_3\ (HO)_2)_n + I_2 + 4O_2 + 2Na^+ + 2H^+$

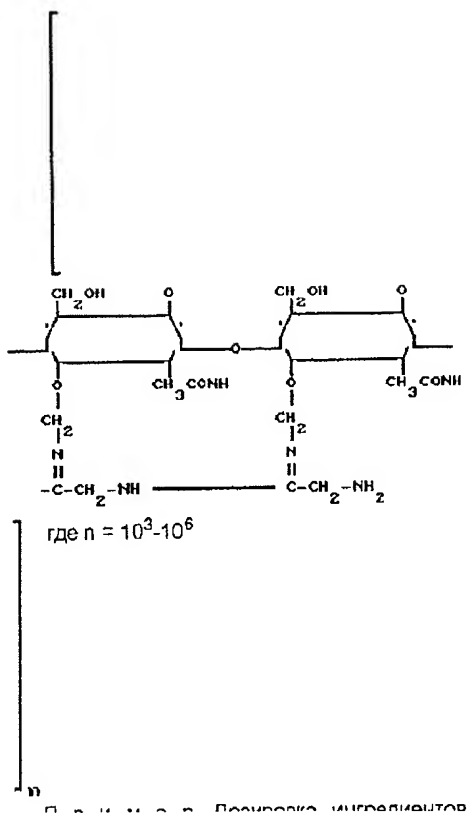
2. $(C_6H_6O_3\ (HO)_2)_n +$

$(C_4H_9N)_n\ (NH_2)_A - (C_{10}H_{17}O_3)_n + 2H_2O$

$\rightarrow$

2

The reaction product corresponds to the following formula:



Where n = 10^3 - 10^6.

Example. (All dosages of ingredients are shown per 1 mg of polysaccharide).

1. Dissolve C-polysaccharide of pneumococcus (pneumococcus antigen; 1 mg) in bicarbonate buffer (0.1 ml; 0.1 M solution; pH = 8). Add 0.1 ml of sodium periodate solution (2 mg/ml) and incubate in the dark over 1 hr. Proceed by adding a drop of glycerol (to remove free sodium periodate; then shake vigorously

at pouring in 0.45 ml of polyethyleneimine solution (molecular mass = 30 to 40 kDaltons) (2 mg/ml) in bicarbonate buffer (0.1 M solution; pH = 8); incubate for 2 hrs.

2. Dissolve a group-specific A-polysaccharide of streptococcus (Group A streptococcus antigen; 1 mg) in bicarbonate buffer (0.1 ml; 0.1 M solution; pH = 8). Add 0.1 ml of sodium periodate solution (2 mg/ml) and incubate in the dark over 1 hr. Proceed by adding a drop of glycerol to remove free sodium periodate; proceed by adding 0.45 ml of polyethyleneimine solution (2 mg/ml)(0.1 M solution; pH = 8) and incubate for 2 hrs in the dark.

Bring the conjugates prepared as described above to 40 ml using a phosphate buffer and pour them into the wells of an enzyme immunoassay tray, 100 µl into each well. Keep the conjugates in such wells over 12 hrs at 4 °C; then rinse with distilled water 4 times any dry at 37 °C. Now the material is ready for immunoassaying.

Due to the fact that the polysaccharide antigen is specific to acute infectious/inflammatory diseases our immunosorbent is suitable for diagnostics of this class of illnesses. Specifically, the sorbent with C-polysaccharide of pneumococcus (pneumococcus antigen) is suitable for diagnostics of diseases caused by pneumococcus (e.g. acute pneumonias); that with A-polysaccharide of streptococcus (Group A streptococcus antigen)

is suitable for diagnostics of illnesses caused by A group streptococcus (rheumatism, tonsillitis, glomerulonephritis); that with Re glycolipid (gram negative bacteria A-antigen) for diagnostics of diseases caused by gram negative enterobacilli (pyelonephritis, enteritis, adnexitis, etc.). For the purpose of medical diagnostics add bull serum albumin (0.5 % solution in phosphate buffer; 100 ml per a well) into the wells of immunoassay tray with conjugate and incubate for 1 hr at 37 °C; then remove the content from the wells and fill them with the serum tested (at 1:5 to 1:640 dilutions; 100 µl a well). Incubate for 1 hr at 37 °C, then rinse 4 times, and add antibodies (100 µl of antibodies per well) to human immunoglobulins conjugated with horseradish peroxidase (at 1:200 dilution); then keep for 1 hr at 37 °C, rinse 5 times and add 100 µl of the substrate (orthophenylenediamine (1 mg/ml; pH = 4.7) containing 0.05 % hydrogen peroxide into the wells. Proceed by keeping in the dark for 40 minutes, then measure optical density at λ = 492 nm.

To illustrate, we provide our examination data for five patients tested with the C-polysaccharide of pneumococcus:

1. Male patient A. Diagnosis: Acute pneumonia. Pneumococcus has been screened from sputum. The titer of anti- C-polysaccharide of pneumococcus antibodies as determined with our invented conjugate as an immunosorbent was equal to 1:320.

2. Male patient B. Diagnosis: Acute pneumonia; bronchial asthma. Pneumococcus has been screened from sputum. The titer of anti-conjugate C-polysaccharide of pneumococcus antibodies as determined with our invented conjugate as an immunosorbent was equal to 1:640.

3. Female patient C. Diagnosis: Pulmonary tuberculosis. The titer of antibodies is equal to 1:40.

4. Male patient D. Diagnosis: Sarcoidosis. The titer of antibodies is equal to 1:10.

5. Male patient E. Diagnosis: Chronic bronchitis. Staphylococcus has been screened from sputum. The titer of anti-C-polysaccharide of pneumococcus antibodies as determined with our invented conjugate as an immunosorbent was equal to 1:20.

The above examples demonstrate that the high titers of antibodies have been observed in patients having pneumococcus-caused illnesses in their acute phases.

For the case of application of the sorbent in solution: add the serum tested (50 µl; diluted by a physiologic saline from 1:1 to 1:1024) to the suspension of sorbent granules with carrier (50 µl). Incubate for no less than 15 minutes; then record the reaction based on formation of color precipitate.

To illustrate the effectiveness of our conjugate we provide our comparative test data. The titers of antibodies have been determined though the known procedure (reference standard) and

with our invented immunosorbent in the patients having streptococcus infections. The data are shown in Table 1.

As follows from Table 1, when using the reference standard it is impossible to assess the degree of reliability at different activities of the process: the data obtained with the standard do not match the process condition. Otherwise, with our invented immunosorbent we have revealed major significant distinctions between the groups of patients. /5

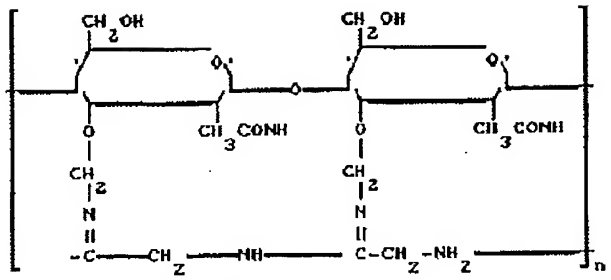
The titers of antibodies have been determined in compliance with our invention and its prototype in parallel. The tests were based on serums of patients from the Examples above. Our data are shown in Table 2.

Based on analytical consideration of data (as shown in Table 2) it has been demonstrated that the prototype sorbent is unsuitable for practice applications due to its low molecular mass and lack of agglutination properties. Otherwise, our invented conjugate may be applied to practice as an immunosorbent in the agglutination reaction.

The Claim:

POLYSACCHARIDE ANTIGEN - POLYETHYLENEIMINE (PEI) CONJUGATE AS AN IMMUNOSORBENT FOR DETECTION OF STREPTOCOCCUS AND PNEUMOCOCCUS INFECTIONS.

A polysaccharide antigen - polyethyleneimine conjugate having a general chemical formula:



где n = 10^3 - 10^6,

Where $n = 10^3 - 10^6$.

The invented conjugate is applicable to practice as an immunosorbent for detection of streptococcus and pneumococcus infections. /6

Table 1

The groups of patients having streptococcus infections	Antistreptolysin-O (reference standard), in units $M \pm m$	Invented A-polysaccharide of streptococcus (Group A streptococcus antigen) recalculated into Antistreptolysin -O units in the dextran agglutination reaction, $M \pm m$	Titers of antibodies in the dextran agglutination reaction, $M \pm m$
1. Expressed rheumatism activity (Stage III) 22 patients	255 ± 89.6 p_{1-2} (no reliable data correlation)	379.1 ± 37.4 $p_{1-2} < 0.001$	1:12 ± 1.2 $p_{1-2} < 0.001$
2. Moderate rheumatism activity (after treatment) 22 patients	219.0 ± 53.6 p_{2-3} (no reliable data correlation)	193.0 ± 14.3 $p_{2-3} < 0.001$	1:6 ± 0.4 $p_{2-3} < 0.001$
3. Beyond acute attacks of the disease (before discharge from the inpatient clinic) 22 patients	188.6 ± 51.7 p_{3-1} (no reliable data correlation)	98.0 ± 10.1 $p_{3-1} < 0.001$	1:3.3 ± 0.4 $p_{3-1} < 0.001$

Table 2

Patients having pulmonary pathologies	Titers of antibodies, immunosorbents	
	Invented immunosorbent	Prototype
1. Male patient A	1 : 320	0
2. Male patient B	1 : 640	0
3. Female patient C	1 : 40	0
4. Male patient D	1 : 10	1 : 5
5. Male patient E	1 : 20	1 : 10